DESOXYRIBOSE NUCLEIC ACID CONTENT IN THE NUCLEI OF SALAMANDER SOMATIC TISSUES

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Irregularities of ploidy in amphibians have been extensively investigated by Parmenter (1933, 1940), Fankhauser and his associates (*cf.* reviews by Fankhauser, 1945, 1952), and Costello and Henley (1949, 1950; Henley and Costello, 1951). These studies have dealt principally with conditions in early embryos and in the epithelium of larval tail-tips. Recently increasing attention has been given to the incidence of endopolyploidy in the adult somatic tissues of vertebrates, of which the mammalian liver and orbital glands have become classic examples (Sulkin, 1943; Biesele, 1944; Swift, 1950; Teir, 1944). The extent of such nuclear deviations in the normal tissues of adult amphibians has received only slight study. Mancini (1945) found a very rare occurrence of large nuclei in the liver of old salamanders. In the renal tubule cells of the Australian desert frog, *Cyclorana,* Dawson (1948) found striking variations in numbers and sizes of nuclei; a similar condition was reported by Schreiber and Melucci (1949) in the South American frog, *Leptodactylus.* In the integumental granular glands of *Triturus viridescens* Dawson (1937) described great volume changes of nuclei associated with secretory activity. Though changes in nuclear size often reflect corresponding changes in chromatin content, this relationship does not invariably hold. Chromosome counts are not feasible in adult tissues showing little or no mitotic activity. Cytospectrophotometry, however, affords a means for measuring the relative content of desoxyribose nucleic acid (DNA) in individual interphasic nuclei and thus provides a valuable technique for the determination of polyploidy and related conditions (*cf.* review by Swift, 1953). The present investigation was undertaken to provide such photometric data for adult tissues of the salamander *Taricha* (*Triturus*) *granulosa granulosa* Skilton. Parenchymal cells of the liver, pancreas, renal tubules, and granular glands of the skin were selected for study.

Methods

Pieces of liver, pancreas, kidney and skin from freshly collected water-inhabiting salamanders were fixed in 30 per cent neutral formalin. Paraffin sections were cut at thicknesses exceeding by two or three micra the average diameters of the constituent parenchymal nuclei. Sections of liver tissue from the same block were placed on all slides as a standard of photometric comparison. Staining with the Feulgen reagent followed the procedure described by Stowell (1945). The time

[1] Facilities for this investigation were provided through grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and through research grant C-2178 M&G from the National Cancer Institute of the National Institutes of Health, Public Health Service.

of hydrolysis in 1 N HCl at 60° C. was 20 minutes, though a difference of five minutes did not produce an appreciable change in the maximal intensity of the color produced by the subsequent treatment with the sulphurous fuchsin reagent for one hour. Unhydrolyzed sections were also stained for controls. All the slides measured were treated with the same stock Feulgen reagent kept completely colorless by storage in tightly closed containers at 5° C.

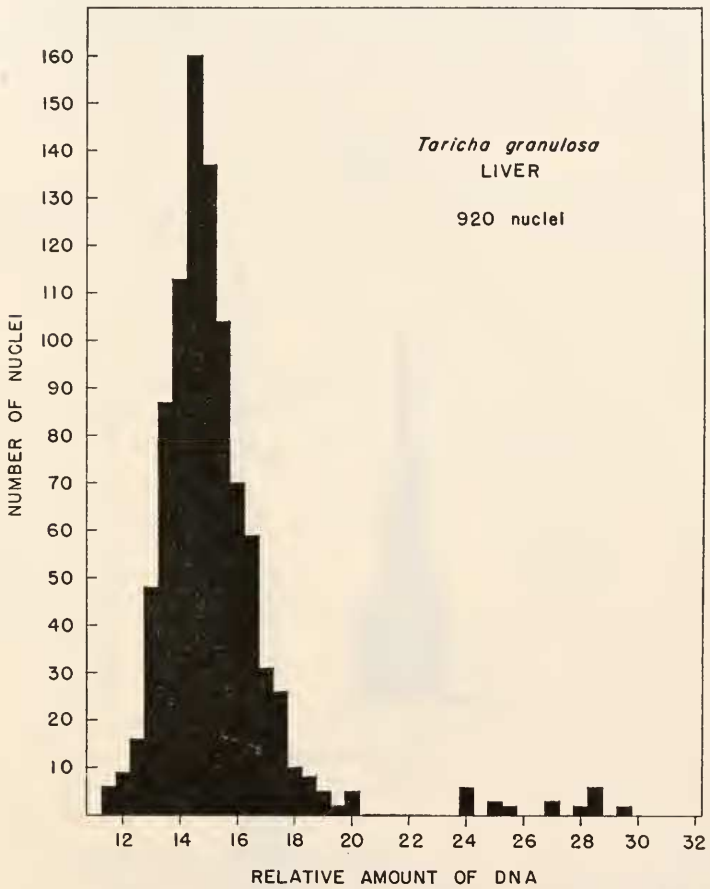

FIGURE 1. Distribution of DNA in liver nuclei of the salamander, *Taricha granulosa*.

Photometric measurements were made with an apparatus similar to that described by Swift (1950). Light of 550 mμ was isolated from a tungsten filament by a Bausch and Lomb interference filter. Optics on centerable mountings were carried on a Leitz microscope stand provided with a side-tube, and consisted of a B. & L. 4 mm. apochromatic objective (N.A. 0.95), a Leitz aplanatic, achromatic condenser (N.A. 1.40) stopped to 5 mm. diaphragm aperture, and 6 × or 8 × Leitz periplan oculars. Transmissions were recorded with a Farrand electron multiplier photometer, employing an RCA 1P21 phototube and a Rubicon galvanometer. The

phototube housing was mounted on a Leitz Aristophot camera. A calibrated iris diaphragm directly under the phototube permitted restriction of the field to any desired area. Measurements of nuclear diameters at predetermined magnifications were conveniently made on the reflex screen of the Aristophot camera.

Care was taken to measure only entire nuclei as determined by critical focusing. Since most nuclei in the present material were approximately spherical, the "plug method" for cytophotometry proved adequate and was used throughout; the largest nuclei of the granular glands, however, were rather irregular in shape, and hence were not included. Plug measurements were based on 60 per cent of the average nuclear diameter. Relative amount of the absorbing substance (DNA) in the whole nucleus was calculated from the formula used by Alfert (1950), *viz.:*

$$A = \frac{E \times r^2}{0.49} \times M^2$$

where A is the amount of DNA in arbitrary units, E is the extinction, r is the plug radius at the phototube level, and M is a magnification factor ($M = \mu$/mm. at the phototube level).

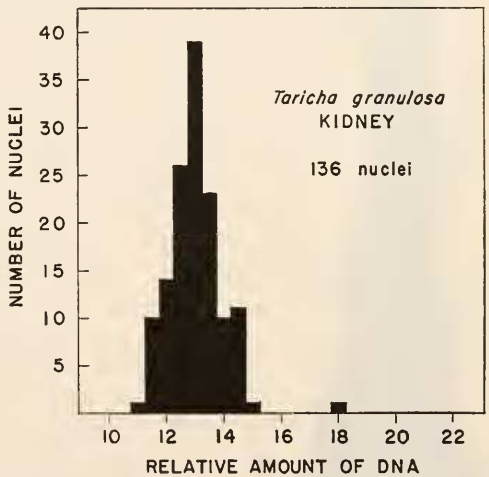

FIGURE 2. Distribution of DNA in kidney nuclei of the salamander, *Taricha granulosa.*

Theoretical aspects of the cytospectrophotometric method and sources of error have been fully discussed by Swift (1950) and Alfert (1950).

In order to obtain a reasonably representative number of determinations for the nuclei of the four tissues, all of the whole nuclei included in any one section were measured, except where deviations of shape did not allow their inclusion, as in the granular glands.

RESULTS

Liver

Interphasic nuclei of liver parenchyma were measured in two animals. The distribution of the relative amounts of DNA was similar in both, so that only one is illustrated (Fig. 1). In this specimen 920 nuclei were measured, in the other

644. Within the ranges 11.5 to 20 and 12 to 20, respectively, the great majority of DNA values formed in both cases a high, rather sharply delimited and fairly symmetrical unimodal curve. A few DNA values, 2.6 per cent of the total number of determinations in the animal figured and 2.2 per cent in the other, were scattered from 24 to 29.5 and from 22 to 33, respectively. The DNA values of liver nuclei were thus grouped into two classes. The peak value for class I nuclei was 14.5 in both specimens; respective means and standard deviations were 14.9 (1.4) and 14.8 (0.9). Class II nuclei, which included DNA values double the class I peak, showed means and standard deviations of 26.5 (2.0) and 25.3 (2.6).

Kidney

The number of kidney tubule nuclei measured was 136 in one animal (Fig. 2) and 129 in another. Unimodal curves similar to those of liver were obtained.

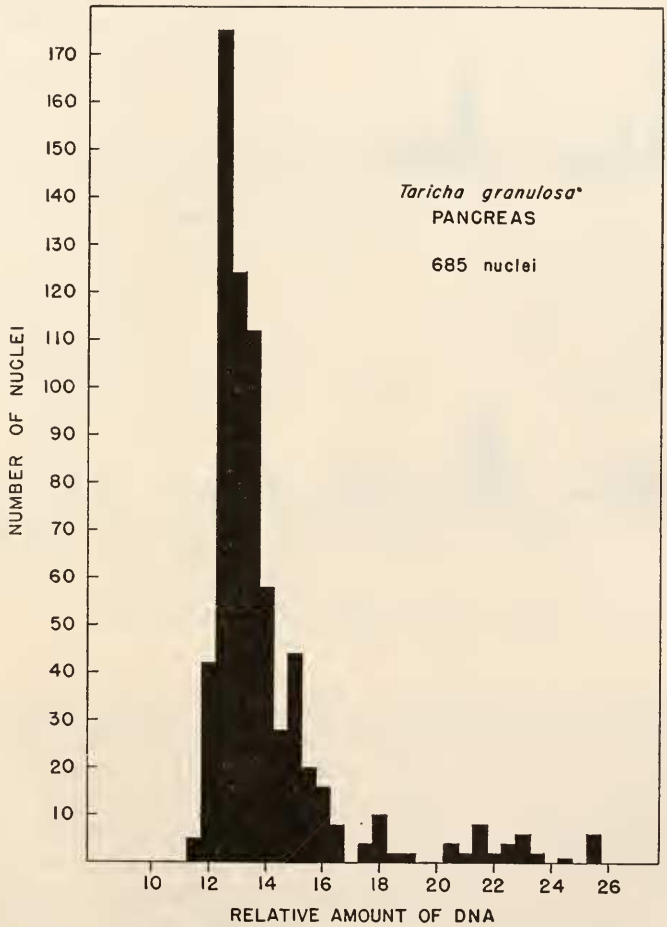

FIGURE 3. Distribution of DNA in pancreatic nuclei of the salamander, *Taricha granulosa*.

In both animals all DNA values ranging from 11 to 18 and from 11.5 to 19.5, respectively, with a peak value of 13.0 in both cases, could be placed into class I; means and standard deviations were 13.0 (0.9) and 13.3 (1.5). In the second specimen two DNA values fell at 21, but none twice the peak amount of class I were found. Careful scanning of several sections did not show any large and darkly stained nuclei which might give class II values. The average DNA content of 13.5 for 10 nuclei of the standard liver section which was mounted together with the kidney sections for comparison, seemed to indicate a slightly lower intensity of stain in the kidney slides. The comparison, however, also showed that class I nuclei of kidney cells were in close agreement with class I nuclei of liver cells with respect to DNA content.

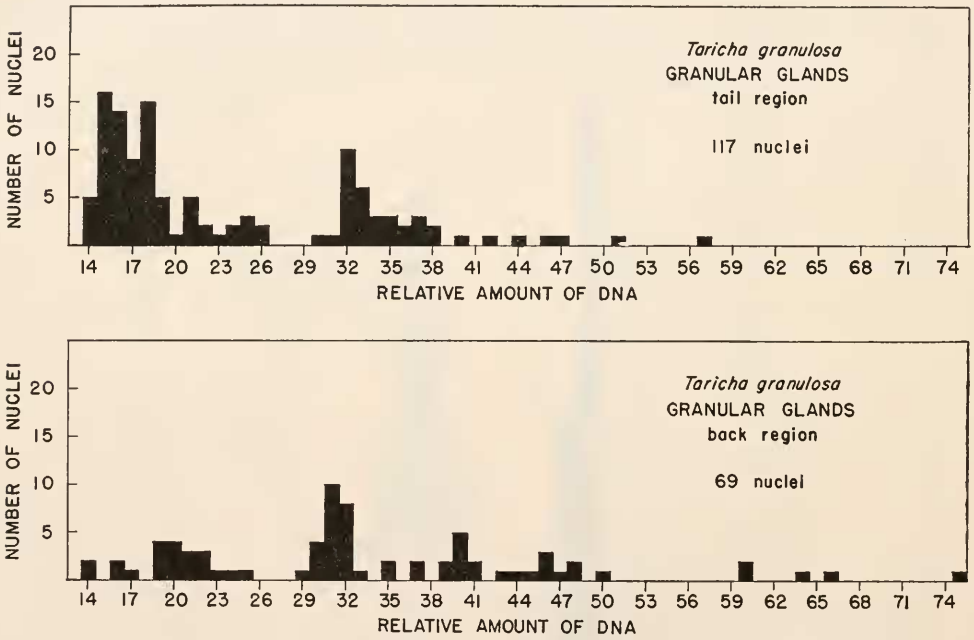

FIGURE 4. Distribution of DNA in the granular gland nuclei of the tail and back regions of the salamander, *Taricha granulosa*.

Pancreas

The distribution of DNA values was determined for 685 nuclei in one animal (Fig. 3) and for 944 in another. The results obtained were essentially similar to those found in liver nuclei. Within the ranges 11 to 19 and 11.5 to 19, respectively, the DNA values formed high unimodal curves (class I nuclei). Only a few values, 5.2 per cent and 1.6 per cent, respectively, of the totals were found in the ranges 20.5 to 25.5 and 20 to 24. The class I peak values occurred at 12.5 in the first animal (mean 13.5, st. dev. 1.3) and at 13.5 (mean 13.7, st. dev. 1.3) in the second. DNA content in the standard liver nuclei averaged 14.7 and 15.0, respectively. The slightly lower mean values of pancreatic nuclei can be accounted

for by their less homogeneous chromatin distribution. Class II means and standard deviations were, respectively, 22.6 (1.6) and 22.9 (1.3).

Granular glands

Skin sections were taken from the dorsal tail region in one animal and from the back region in another. In the first case most of the granular glands were at earlier developmental stages than in the second. In the younger glands the cell membranes were well defined, the cytoplasm was evenly filled with secretion granules, small nuclei were common, and most of the larger nuclei occupied a central position in the cell. A larger number of mature glands were found in the second location. In these the cell boundaries were lost (apocrine secretion), the cytoplasm was vacuolated, and peripherally located giant nuclei were numerous. These very large nuclei were usually flattened and could not be measured with the present photometric method. Because of this limitation only a small number of measurements were possible, 117 in the first animal and 69 in the second. Therefore the results, graphically shown in Figure 4, include only a partial representation of the granular gland nuclei.

A wide spectrum of DNA values was obtained in both specimens. In the tail region, where the smaller nuclei were most numerous, low DNA values were in the majority. In the back region, larger nuclei with high DNA values were relatively more abundant. Delimitation into nuclear classes is difficult. A possible grouping would include in class I the values 14 to 26, in class II 29 to 51, and in class III 57 to 75. Such a break-down would fit both graphs and would roughly yield successive doubling of mean values (I = 17.7 and 19.7; II = 35.5 and 36.5; III = 57 and 65). However, in comparison with the control liver sections, which showed means of 14.3 and 14.2, respectively, the above class I means are rather high. In part, this may be accounted for by the very homogeneous distribution of chromatin in granular gland nuclei, resulting in higher extinction values. If class I is taken to include only values 14 to 18, the means fall around 16 (in better agreement with the liver values), with doubling at 32 (at which peak groups occur in both graphs) and quadrupling at 64 (in harmony with the highest plots). This would leave considerable numbers of nuclei with intermediate values, notably the groups between 20 and 26 and between 39 and 51. Further consideration of this situation is deferred to the discussion.

Discussion

The nuclear DNA classes indicated by the distribution curves suggest a progressive doubling of chromatin. Class II nuclei might be interpreted as interphasic doubling prior to mitotic separation of diploid complements were it not for the fact that no mitoses were observed in the tissues examined. Hence it is more likely that these nuclei are definitively tetraploid or in an equivalent polytene condition. This conclusion gains strength in the case of the granular glands where class III (octoploid) values occur. The general picture is in agreement with similar phenomena in mammalian and other tissues (see Swift, 1953).

Variation within classes may be due in part to over-all error in the photometric technique, the exact value of which cannot be assessed but probably does

not exceed 15 per cent (Swift and Kleinfeld, 1953). Some of it might be accounted for by incomplete synthesis of DNA during endomitotic activity. The possibility of aneuploidy also cannot be disregarded, since its occurrence in the somatic tissues of mammals has been reported by Therman and Timonen (1951) and by Hsu and Pomerat (1953); Walker and Boothroyd (1954), however, believe that its incidence has been overestimated. In the absence of actual chromosome counts, the presence of aneuploid nuclei cannot be established.

The large number of individual nuclei measured in the several tissues of the present study permits estimation of polyploid frequencies. Unlike certain mammalian tissues, where polyploidy has been found to be common in beef liver and may even reach 50 per cent in old mouse liver, as reported by Swift (1950), salamander hepatic and pancreatic nuclei show very low polyploid frequency. Only about 2.5 per cent tetraploidy (class II nuclei) was found in the liver, 5.2 per cent in the pancreas, and no octoploid amounts in either. Kidney nuclei were entirely of the diploid class. The polyploid frequency, however, might vary with age of the animal, as is known to happen in mammals. Seasonal variation might also occur.

The granular glands of the integument present a case of special interest. The conspicuous nuclear changes in size, position and shape accompanying the various phases of secretory activity have been fully described by Dawson (1937) for the salamander *Triturus viridescens*. He thought, though the evidence was not conclusive, that these changes might suggest nuclear participation in the glandular activity. In the mature glands he noted nuclei of gigantic size, alveolar patterns of chromatin distribution, vacuolizations, and indications of possible passage of nuclear material into the cytoplasm.

In the present study, since a great part of the nuclear population could not be measured because of various shape irregularities, the account of the DNA distribution is not complete. There is, however, sufficient evidence that high polyploid (and possibly aneuploid) frequency exists, and an indication that it increases in degree as well as frequency during the development and maturation of the glands. This is exemplified by the greater prevalence of high DNA values in the older glands of the back region. Though only a few determinations were technically possible for nuclei in the octoploid range, large darkly staining and irregular or flattened nuclei, which might give similar or higher values, were frequently encountered. Thus it was not possible to establish the full extent of polyploidy in the mature glands. Giant nuclei which stained lightly were also observed, but their irregular shapes likewise precluded photometric determination by the present method.

In view of the considerable number of nuclei with apparent intermediate DNA values, it may be that chromatin multiplication is not wholly euploid. As already pointed out, mitoses are absent, so that direct evidence through chromosome counts could not be obtained. If polyteny is involved, it is conceivable that chromonemal duplication may be uneven. Or it may simply be that the intermediate values reflect a high rate of endomitotic activity, resulting in a picture similar to that obtained for tissues with high mitotic rates.

A point of additional interest attaches to the relation between volume and DNA content in the granular gland nuclei. This is graphically shown in Figure 5 which includes the data from both tail and back regions. Each plot shows the average

DNA content for all nuclei of the same size. It is seen that nuclear volumes increase regularly with DNA content. It may be observed, incidentally, that DNA values intermediate between class peaks are associated with intermediate nuclear volumes, which suggests that the error in photometry cannot be great.

Leuchtenberger and Schrader (1952) found that in the salivary gland of the snail *Helix* DNA content likewise varies directly with nuclear size, though both decrease with the production of cytoplasmic secretion. No loss of DNA accompanying secretory activity has been found in the granular glands. In this respect the granular glands resemble the pharyngeal and thoracic glands of honeybees, in

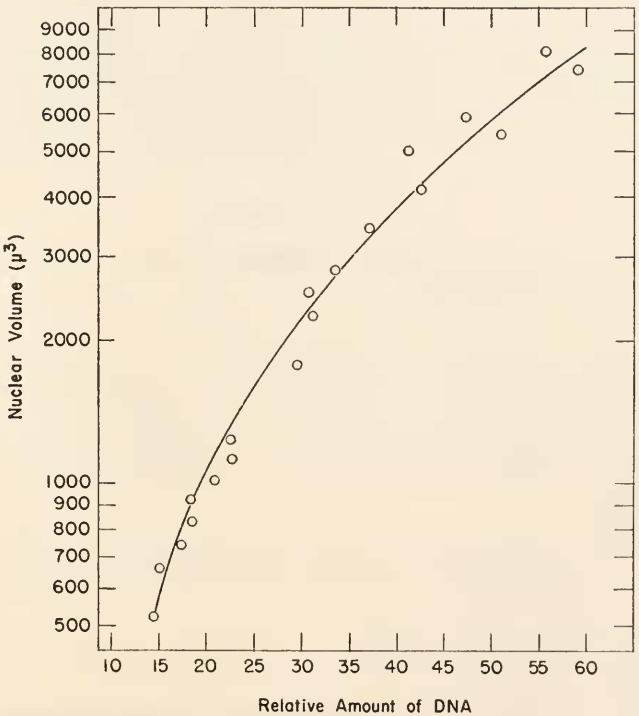

FIGURE 5. Relation between volume and DNA content in the granular gland nuclei of the salamander, *Taricha granulosa*.

which Merriam and Ris (1954) noted a positive correlation between degree of ploidy and secretory activity.

SUMMARY

1. Relative DNA content was determined photometrically for individual Feulgen-stained parenchymal nuclei of the liver, kidney, pancreas and granular glands of the salamander *Taricha* (*Triturus*) *granulosa granulosa* Skilton. Graphs of these values indicated the presence of nuclear classes falling into polyploid ratios.

2. A total of 920 liver nuclei were measured in one animal and 644 in another. A vast majority of the DNA values fell into the first class, representing the

diploid condition. Only 2.6 per cent and 2.2 per cent, respectively, of the total number of nuclei constituted class II, which included values roughly double those of class I. No higher values were found.

3. In the kidney tubules, 136 and 129 nuclei were measured. All could be included in class I.

4. In the pancreas 685 nuclei were measured in one animal, 944 in another. Only 5.2 per cent and 1.6 per cent, respectively, of the total number of nuclei fell into the range of tetraploid values. No higher values occurred.

5. Degree and frequency of polyploidy was marked in the granular glands of the integument. Diploid, tetraploid, and octoploid values were obtained, and presumably higher values occurred in very large nuclei too irregular in shape for photometric measurement by the method employed. Polyploid nuclei increased in frequency with maturation of the glands and exceeded diploid types in number. A considerable number of nuclei with intermediate values suggested the occurrence of aneuploidy or of incomplete DNA replication incident to high endomitotic activity. A regular increase in nuclear volume accompanied rise in DNA content.

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